THE GUMMOSIS OF THE SUGAR-CANE.

(*Bact. vascularum*, Cobb).

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(Plates iv.-v.)

Gummosis is a disease pretty widely distributed among plants, and is recognised by an unhealthy appearance of the plant as a whole, by the leaves dying or rotting, and especially by the presence of a yellowish gummy matter in the vessels. On cutting across the vessels of a badly diseased plant, such as the sugar-cane, small yellow viscid drops of gum quickly gather on the cut ends of the vascular bundles. In slightly diseased plants the microscope may be necessary to demonstrate the gum plugging the vessels. The disease has been specially noted in the sugar-cane, the sugar-beet and the vine chiefly on account of the economic importance of these plants, but it is not unknown among other members of the vegetable kingdom, as for example the fig, olive, mulberry, potato, carrot, and tomato.

The gummosis of the vine has been ascribed to the activity of a bacterium by Prillieux et Delacroix,* but Mangin,† and also Rathay,‡ consider that the formation of gum in the vessels by bacteria is very problematical. Indeed, Mangin goes further and says that the bacteria live upon the gum.

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* Prillieux et Delacroix, Cent. f. Bakt., 2 Abt., i., 300, Ref.

†Mangin, *op. cit.* and also *ibid.* ii., 621, Ref.

‡Rathay, *ibid.* ii., 620, Ref.

The gummosis of the sugar-beet has been investigated by many. Among others, Arthur and Golden separated a bacterium which, when inoculated into sound beets, produced the disease in some cases. Busse* also investigated the disease, and concluded from his own experiments and from those of others that there is a group of bacteria which cause the gummosis of the beet. He experimented upon sound beets with pure cultures, and found that after a period of growth the infected roots had developed a greater or less gummosis. The bacteria that are claimed as being the exciting cause of gummosis of the beet are all capsulated, non-sporulating organisms, and it would appear that the gum is the capsular envelope.

The Gummosis of the Cane.

The bacteriology of the gumming of the sugar-cane has been investigated by Cobb,† who invariably found microbes in the gum of diseased stalks. He inoculated the gum into tubes of agar and gelatine and obtained bacterial growths. The cultural characters of the organism are described as follows:—"Cultures on agar-agar gave at first roundish colonies on the surface having to the unaided eye no structure, either radiated or concentric. In the course of a few weeks the yellowish white and somewhat opalescent growth had extended several inches along the edge of the tube on the surface of the agar-agar and *between* the tube and the agar-agar without causing any liquefaction. On gelatine the growth was much slower, and remained circular and almost imperceptibly concentric. Its colour, &c., were as on agar-agar." The italics are in the original.

It is unfortunate that the only information regarding the growth characters which enables the organism to be identified is so limited. The description would apply to at least eleven described non-liquefying species of bacteria. The characters,

* Busse, Cent. f. Bakt., 2 Abt., iii., 680, Ref.

† Cobb, Agric. Gazette of N.S. Wales, 1893.

however, enabled Cobb to recognise it as a new species, to which he gave the name *Bac. vascularum.*

In an investigation into the pathogenicity of the organism Cobb* inoculated five apparently healthy canes with the gum and retained one as a control. Unfortunately the control cane was lost, and of the others four were gummed and one died. In the four stalks that were gummed, the gummosis was most marked in the neighbourhood of the point of inoculation. But as all the canes in the stools developed gummosis there is the probability that the inoculated canes would also have become affected independently of the inoculation. The fact that gummosis was most marked in the neighbourhood of the site of inoculation indicates that the gum probably set up gummosis; but like the majority of plant infection experiments the result of Cobb's experiment is not decisive. Even if the experiment had been most satisfactory it would only have shown that cane-gum could produce gummosis, and thus the pathogenicity of the bacterium itself would remain doubtful. In all infection experiments the recognised and legitimate method is to purify the bacterium by growth in plates of artificial media, and after it has been proved that the bacteria are undoubtedly pure by their constant appearances of growth, etc., upon one medium, to employ the pure culture for purposes of inoculation.

The Method of Research.

The cause of the gummosis of the cane, therefore, appeared to be sufficiently undecided to warrant a thorough investigation, and I decided to attack the problem from the side of the gum rather than the plant—a method that has not been previously tried, and one which promised to give more conclusive results than could be hoped for with infection experiments. If a gum could be produced in the laboratory from a bacterium isolated from the cane, and if it were identical with the gum obtained from diseased canes, then the bacterial origin of the gum and the

* Cobb, Agric. Gazette of N.S. Wales, 1894.

identity of the organism would be placed beyond all doubt. The question, then, as to the gum being a pathogenic secretion of the plant itself would be answered conclusively in the negative.

The Isolation of the Organism.

To facilitate the investigation the Colonial Sugar Refining Co. offered me a portion of their small supply of cane-gum which had been collected some years ago. Furthermore, one of their officers, Mr. Morison, inoculated tubes of media in the cane fields with gum exuded from diseased canes. As, however, I received several specimens of gummed cane, the tube cultures which did not contain the desired organism need not be further noticed. The specimens of diseased cane were the variety known as Rappoe, and, on being cut across, small yellow viscid drops exuded from the ends of the cut vessels. By using a lens of low power (× 10) minute portions from the centre of exuded drops of gum were abstracted and inoculated into various media, which were placed under different conditions of culture. It need scarcely be said that the cane was cut and the infections made with the usual precautions as regards sterilising the outer surface of the cane, knives, etc.

Under anaërobic conditions there was no growth either in fluid or on solid media. On slices of cane and in acid fluids such as wort and cane-juice no bacteria developed. In other neutral fluids the bacteria grew scantily. On agar and gelatine in the presence of cane or fruit sugar bacteria grew slowly at temperatures of 30° C. and under; at 37° and over no growth could be obtained under any condition whatsoever. What appeared to be the most suitable medium for the growth of the gum bacteria was neutral cane-juice gelatine. The next best media were ordinary glucose gelatine and slices of potato. From the growth on cane gelatine a bacterium was separated in the pure state by repeated cultivation upon plates of cane gelatine, and its cultural characters were studied upon other media. The appearances of the cultures are given at the end of this paper, and they will serve to identify the organism.

Compared with other bacteria the organism is of slow growth. On suitable solid media it forms raised yellowish patches, which have the appearance and consistency of softened yellow bees'-wax, and when treated with water the culture slowly mixes, forming what appears to be a partial suspension and partial solution. In stroke culture on cane-gelatine the growth collects in places along the stroke, and forms characteristic hanging drops ("tear-drops"). The gelatine is very slowly liquefied, the liquefaction being best seen by the disappearance of the gelatine under the slime, which gradually collects as the culture grows, and gravitates to the bottom of the sloping gelatine.

The Bacterial Gum or Slime.

When a pure culture of the bacterium is smeared over the surface of neutral cane-gelatine or agar in a suitable vessel, and incubated, a luxuriant growth is obtained after the lapse of about a week. The culture is precisely similar in appearance and consistency to cane-gum. Both have the same soft, buttery appearance and gummy consistency, and both slowly mix with water to form a pale yellow opalescent solution. The opalescence is due to the suspended bacteria, from which it is rather difficult to separate without at the same time removing the gum. The separation cannot be effected by filtering through paper, and the usual coagulating agents coagulate both gum and bacteria. Aluminium hydrate, for example, forms an insoluble compound with the gum. An attempt was made to clarify the suspension by heating it up to three atmospheres in the autoclave, a proceeding which was successful with another gum-forming bacterium, but the suspension was unaltered. Eventually the bacteria were separated by filtration through porous porcelain. A clear solution of the gum was thus obtained, but at the same time it was noted that a considerable amount of the gum remained on the porcelain, adhering to the bacteria, even after the suspension had been boiled for some time to diffuse the gum. The filtered and clear solution was tested simultaneously with an opalescent suspension, and it was found that the two behaved similarly on the addition of the various reagents, from which we must con-

clude that in the suspension the bacteria are inert, and for purposes of identification of the gum it is unnecessary to separate them.

In testing the bacterial slime and the cane-gum a number of reagents suggested themselves, and the results of the numerous tests which identify the two are shown in the following table. In considering the tests it must be borne in mind that the cane-gum at my disposal was very limited, and the solutions consequently were weak, which accounts for the absence of perceptible precipitates on the addition of dilute acetic acid and a mixture of acetic and tannic acids.

THE CHEMICAL REACTIONS OF THE GUM FROM CANE AND OF THE BACTERIAL SLIME FORMED IN LABORATORY CULTURES.

	Cane-Gum.	Bacterial Slime.
Lead acetate	Precipitate.	Precipitate.
Basic lead acetate	,,	,,
Ammoniacal lead acetate ...	,,	,,
Barium hydrate	,,	,,
Calcium hydrate	,,	,,
Copper sulphate	,,	,,
Ferric chloride	,,	,,
Hydrochloric acid	Opalescence.	Opalescence.
Alcohol	Slimy voluminous ppt. in presence of salts.	Slimy voluminous ppt. in presence of salts.
Aluminium hydrate	Coagulation.	Coagulation.
Dilute acetic acid	No precipitate.	Opalescence.
Glacial ,, ,, ...	Precipitate.	Precipitate.
Acid mercuric nitrate	Ppt. soluble in excess; no colour reaction.	Ppt. soluble in excess; no colour reaction.
Xanthoproteic reaction ...	Colour reaction.	Colour reaction.
Sulphuric and phosphotungstic acids	Precipitate.	Precipitate.
Acetic and tannic acids ...	Opalescence.	Opalescence and precipitate.
Acetic acid and potas. ferrocyanide	Opalescence.	Opalescence.
Hydrochloric acid and potas. mercuric iodide	Ppt. on standing.	Precipitate.
Sodium hydrate and copper sulphate	No biuret reaction, but ppt.	No biuret reaction, but ppt.

No reactions were obtained with KI_3, $AgNO_3$, KOH, $BaCl_2$, NaOH, conc. HCl, picric acid, Adamkiewicz reagent.

The precipitate obtained on the addition of alcohol to a suspension of the gum or slime in water is exceedingly voluminous; a precipitate which occupies a volume of about 50 c.c. becomes, after squeezing out the dilute alcohol in a calico strainer, a small pellet measuring about a quarter of a c.c. On precipitating the aqueous suspension three or four times with alcohol an opalescent alcoholic solution is obtained, from which the gum can be precipitated by small quantities of neutral salts, such as sodium chloride. This fact, together with the reactions obtained with some of the albuminoid reagents, suggested the similarity of the gum to the mucins. That the zoogloea slime of bacteria consists of mucin, or a substance nearly allied to it, has already been suggested, but there are many points of difference between the bacterial slime and the mucins. The crude gum obtained by precipitating the cultures with alcohol contains 6·1% of ash and 3·08% nitrogen in the ash-free, dry substance. Repeated precipitation with alcohol, and also filtration of the gum from the accompanying bacteria, would undoubtedly lower this percentage. It is, therefore, apparent that the nitrogen content alone is sufficient to distinguish the slime from mucin or the allied mucinoids.

The identity of the bacterial slime with cane-gum proves conclusively that the isolated bacterium is the direct cause of the gummosis of the plant, and also that the gum is no secretion of the plant upon which the bacteria live saprophytically. Since this applies to the gummosis of the sugar-cane, it probably also applies to the gummosis of the vine and other plants, in which case Mangin and Rathay are wrong in considering that the action of the bacteria in causing gummosis of the vine is very problematical. Cane-gum is nitrogenous, and when a clear filtered solution is exposed to the air it speedily produces a luxuriant growth of foreign organisms. These undoubtedly feed upon the gum, and it is possible that this observation gave rise to the idea that the bacteria in the vessels of the plant live upon the gum.

The Conditions of Gum-Formation.

Having proved the relationship of the gum to the bacterium, there remained the determination of the conditions under which the gum is formed. The slime had been first obtained in quantity by growing the bacteria upon cane-juice agar, *i.e.*, a solution of 4% of agar added to an equal volume of neutralised cane-juice; and although the growth was very good, yet there appeared to be room for improvement. The best medium is undoubtedly very faintly acid cane-juice gelatine, but, unfortunately, this cannot be employed when the slime is wanted for chemical investigation, because the medium is slowly liquefied. The first experiment was made upon cane-juice agar, and had for its object the question of the improvement of the agar by peptone and, incidentally, the effect of a temperature of 30° as against 22° C. Previous tests had shown that no growth occurred at 37°. In this and the following experiments the numbers indicate the order of merit of the cultures at the time of observation ; 1 being the most luxuriant growth, 2 the next best, and so on.

The Effect of 1% Peptone on Cane-agar Cultures at 22° and 30° C.

	2 days.	4 days.
1% peptone at 22°	2	2
,, ,, 30°	1	1
No ,, 22°	3	3
,, ,, 30°	3	4

This experiment showed that more slime was formed when the cane-agar contained 1% of peptone, and when the culture was incubated at 30°. There, however, is the doubt that 1% peptone is too much, and to test this point various quantities of a 10% peptone solution were added to 10 c.c. portions of a faintly acid medium containing glucose 10%, sodium phosphate 0·2%, potassium chloride 0·5%, agar 2%, and the bacteria were inoculated upon the sloped surface of the portions. Cane-agar was not used on account of the unknown nature of the constituents.

THE INFLUENCE OF PEPTONE.

Temperature = 30° C	2 days.	4 days.	6 days.	8 days.
No peptone	5	3	4	6
0·1 c.c. 10% solution = 0·1% ..	4	2	1	1
0·2 ,, ,, ,, 0·2% ...	4	2	1	1
0·5 ,, ,, ,, 0·47% ..	2	1	1	1
0·7 ,, ,, ,, 0·65% ...	2	1	1	1
1·0 ,, ,, ,, 0·91% ...	3	1	1	2
2·0 ,, ,, ,, 1·66% ...	1	1	2	3

It appears from these results that about 0·5% of peptone in the medium is a good quantity to employ. With a greater percentage the growth starts sooner, but as time goes on it becomes slower, and is ultimately surpassed by the lower percentages.

The reaction of the medium best suited to the bacterium was also investigated, and for this purpose various quantities of 10% tartaric acid and 10% sodium carbonate solutions were added to 10 c.c. portions of neutral medium, containing saccharose 5%, peptone 0·5%, potassium phosphate 0·5%, agar 2%.

THE REACTION OF THE MEDIUM.

Temperature = 30° C.	2 days.	4 days.	6 days.	8 days.	10 days.
Tartaric acid 0·01% ..	1	1	1	1	1
,, ,, 0·02% ...	2	2	2	2	1
,, ,, 0·03% ...	—	3	3	3	2
,, ,, 0·05% ...	—	—	—	—	—
,, ,, 0·08% ..	—	—	—	—	—
Sodium carbonate 0·01%	—	4	6	7	8
,, ,, 0·02%	—	5	7	8	—
,, ,, 0·03%	—	—	—	—	—
,, ,, 0·05%	—	—	—	—	—
,, ,, 0·08%	—	—	—	—	—
Neutral control tube ..	—	4	5	6	5
,, ,, ...	—	4	5	6	5

The effect of slight differences of acid or of alkali in the culture media is very pronounced. In an absolutely neutral medium the bacteria grow very slowly, while, when the reaction is faintly acid, the growth is quick and luxurious. Alkalies prohibit the

multiplication of the microbe. With 0·02% the stroke was slow to show itself, was always very scanty, and finally, after ten days' incubation, it dried up.

The Location of the Bacteria.

Cane-juice has an acidity equal to an amount of tartaric acid varying from 0·2 to 0·3%, and as the juice is derived from the tissue we can understand why the bacteria avoid these strongly acid tissues and inhabit the slightly acid or neutral fluids of the vascular system.

In view of the fact that in many bacterial diseases of plants, the microbes are found only in the vessels, and that the bacteria are very sensitive to the reaction of the contents of the vessels, it would appear that the immunity of plants disease-proof to bacterial infection may depend upon a relatively greater acidity or alkalinity of the vascular contents as compared with susceptible varieties.

A peptonised medium similar to that used in testing the influence of peptone was employed in a small test to corroborate the general conclusion that had been drawn during the work upon the bacterium with regard to the temperature, and from the growths upon the agar it was manifest that a temperature of 30° C. was best suited to the organism.

The Optimum Temperature.

	2 days.	4 days.	6 days.
22°	2	2	2
25°	3	2	2
30°	1	1	1

That the bacterium grows rapidly at 30° C. (86° F.) and not at all at 37°, serves to explain in part at least why the disease occurs more frequently in cold, wet seasons. Such seasons check the growth of the plant, the vitality is lowered, and at the same time the bacteria, being under favourable conditions of tempera-

ture, grow rapidly, and clog the vessels, on account of which the plant is unable to obtain its food supplies, and soon succumbs.

One point to be decided was the influence of salts upon the growth of the bacterium, and to test this matter a faintly acid medium containing peptone 0·5%, dextrose 10%, agar 2%, was prepared, and about one-quarter of a gramme of salt was added to each 10 c.c.

The Influence of Salts.

	2 days.	4 days.	6 days.	8 days.
Potassium nitrate	3	1	2	2
,, monohydrogen phosphate	2	1	1	1
,, dihydrogen phosphate ...	5	—	—	—
,, sulphate	2	1	2	2
,, chloride	3	1	2	2
,, citrate	1	1	2	2
,, sodium tartrate	4	2	4	3
Sodium chloride...	5	4	6	6
Ammonium chloride	3	1	2	2
Calcium chloride	2	1	2	3
Magnesium sulphate	3	1	2	2
Check (no salts)...	2	2	4	3

This experiment shows that (1) the bacterium has a preference for phosphate and is indifferent to the other acid radicles ; (2) the acid potassium phosphate, on account of its acidity, checked the growth; (3) potassium may be replaced by calcium, magnesium or ammonium ; (4) sodium is a distinct poison.

The Poisonous Action of Common Salt.

After noting the poisonous action of sodium salts, the idea naturally suggests itself that common salt might be advantageous in checking the gummosis of the cane. Common salt, however, is a poison for most of the higher plants, and might injure the sugar-cane if used in doses sufficiently great to inhibit the growth of the bacterium. On enquiry, I find that healthy crops of cane are grown in Fiji upon soil containing anything under 1% of common salt, although when this amount is exceeded t h

crops are not so healthy. This would lead one to expect favourable results with salt when applied to fields containing canes liable to the disease.

I have enquired if canes grown on the coast, and within reach of the salt spray become diseased to the same extent as plants grown inland, and have been informed by Mr. T. U. Walton, B.Sc., of the Colonial Sugar Refining Co., who made the necessary enquiries, that the majority of the managers of the different mills consider that canes grown upon soils which are known to be pretty well impregnated with salt through being subject to the influence of tidal waters, are not less liable to be affected with gummosis than crops upon other soils. The opinion is that they are more prone to develop the disease, but with regard to this it must be borne in mind that such soils are generally badly drained, and it is to the defective drainage that the prevalence of the disease is ascribed. Cases can be quoted in which gummosis has been remedied by the improvement of the land by draining and the planting of new seed cane.

In direct contradiction to the other officers, one of the managers thinks that it may be safely said that on lands adjacent to the sea beach, where the soil is contaminated with salt, gummosis has not been nearly so bad as in districts distant from the sea. He instanced the case of a man, whose farm was on one of the islands in the Lakes, Clarence River, being able to grow a comparatively sound crop of Mauritius Ribbon long after this variety of cane had been abandoned by everyone else on account of its tendency to develop gummosis. A large portion of his farm was submerged by King tides.

The Colonial Sugar Refining Co. in the following season intend to test the action of common salt upon perfectly drained soils.

The presence of salt in the cane is preferable to gum, inasmuch as the former collects in the molasses and does not otherwise make its presence evident, while the gum, besides diminishing the crop, interferes with the crystallisation of sugar.

Although one might by experiment find a substance that would inhibit the disease, the best practice is, undoubtedly, to

grow disease-resisting varieties, as has been previously recommended, not only for gummosis, but for other diseases. In this relation it may be mentioned that there are one or two well known varieties which have never been known to develop gummosis. The best known of these sound varieties is Tanna, a stout heavy cane, hard in the rind and containing a high percentage of fibre. The sugar content is, however, not so high as it is in some of the varieties which are liable to gummosis. Fields which have yielded badly diseased crops, have, when planted with Tanna, produced perfectly sound canes.

In order to form a gum as it undoubtedly does, the bacterium probably requires a carbohydrate, and although saccharose has been employed and found exceedingly useful, yet it does not follow that it is necessary, or that saccharose is the best carbohydrate. Other sugars might give a better yield of gum. To test this question, about half a gramme of various substances was added to 10 c.c. portions of a faintly acid medium containing peptone 0·5%, sodium phosphate 0·2%, potassium chloride 0·5%, agar 2%, and after sterilisation of the tubes, the sloped surfaces were inoculated and then incubated.

THE INFLUENCE OF CARBOHYDRATES, ETC.

Temperature = 30°	2 days.	4 days.	6 days.
Glycerine	—	—	—
Starch	—	—	—
Dextrin	—	—	—
Dextrose	1	2	3
Levulose	1	1	1
Saccharose	1	1	1
Maltose	—	—	—
Lactose	—	—	—
Blank test	—	—	—

This experiment showed that either dextrose, levulose, or saccharose are absolutely necessary for the free growth of the organism and the production of gum. The bacteria did not grow in the presence of the other sugars and carbonaceous

matters, from which we infer that these cannot be utilised. Of the three sugars, levulose and saccharose are more easily assimilated than dextrose.

The organism does not secrete invertase. This was shown by the practical absence of invert sugar in the agar cultures. In testing inversion fluid cultures are preferable, but as this bacteria does not grow to any extent in saccharose fluid media, an agar culture had to be taken. The agar was melted and dissolved in water, after which the solution was treated with basic lead acetate, etc., as is customary in sugar determinations. Of the saccharose in the tube originally, only 3% had been inverted to fruit sugar, a quantity which might easily have been produced through the hydrolytic action of the small quantity of acid in the medium.

The Most suitable Medium.

From these experiments we conclude that an excellent medium for the growth of the bacterium and the production of gum would have the following composition:—

Peptone	0·5
Saccharose or levulose	5·0
Potassium phosphate	0·5
Agar	2·0
Tap water	100

Acidity of 10 c.c. = 0·14 c.c. tenth-normal acid.

On this saccharose medium the bacteria grew most luxuriantly at the optimum temperature (30°), and since the organisms grow so well, we cannot doubt that similar conditions as regards nutrition, acidity, and temperature favour the development in diseased canes.

Bacterium vascularum, Cobb.

Shape, &c.—The organism appears as an actively motile, short rod, and when stained and imbedded in balsam has an average measurement of 0·4 : 1 μ. Carbol violet followed by dilute alcohol produces the best films; fuchsin stains the gum, which

usually adheres more or less to the cells; the blues stain but feebly. By using the night-blue method with the scanty growth obtained on ordinary agar the flagella can be easily stained. They are single and terminal. The bacteria are not coloured by the Gram method of staining. Spores were not obtained and are probably never formed.

Relations to oxygen and temperature.—It is a strong aërobe, and grows best at 30°; at 37° there is no growth.

Ordinary glucose-gelatine plate.—The colonies develop slowly. In 7 days at 22° they are 1 mm. in diameter, and appear as small, raised, viscid drops. When magnified 60-fold they appear round and uniformly granular, like a thin yeast colony; the deep colonies are like those upon the surface. In 20 days the colonies reach a diameter of 4-8 mm., and look like drops of yellow bees'-wax. The medium shows no sign of liquefaction, but when the colony is scraped or washed off a pit is revealed.

Ordinary gelatine plate.—The colonies grow as in the presence of glucose, but much more slowly.

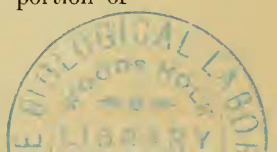
Ordinary gelatine stroke.—The growth is scanty, narrow, flat, and ivory-white in colour. It slowly gravitates to form a yellowish-white mass. The medium under the stroke is depressed.

Neutral cane-gelatine stroke.—There is formed a characteristic convex, deep yellow stroke, with waxy drops at intervals, and at the base. The gelatine in contact with the culture is slightly liquefied in three weeks. The colour, bloom, and general appearance is that of yellow wax, or of a mixture of yellow vaseline and paraffin. The "tear-drop" appearance of the stroke is characteristic.

Neutral cane-gelatine stab.—The growth forms in the upper portion of the stab only in a filiform manner, and forms a hemispherical, deep yellow nail-head. No gas bubbles are produced.

Ordinary glucose-gelatine stab.—As cane-gelatine, but neither so luxuriant nor so deeply coloured. No gas formation was observed.

Ordinary gelatine stab.—Filiform growth in upper portion of stab, with small, raised, white, glistening nail-head.



Ordinary nutrient-agar stroke.—A slow-growing thin, yellowish white glistening stroke.

Glycerine nutrient-agar stroke.—A thin, broad, translucent white, moist, glistening growth, with turbid condensed water. The colour deepens to a primose-yellow.

Saccharose (10%)-peptone (0·1%)-agar.—A thin white fluid growth, which gravitates into the condensed water, in which there is a yellow sediment.

Potato.—A primrose-yellow, moist, glistening growth, sometimes raised and restricted, at other times flat, watery, and spreading over the surface. Compared with agar or gelatine, the growth is rapid.

Carrot.—A raised, slimy, yellow growth, at first restricted, but eventually covers the surface and gravitates.

Turnip.—As on carrot.

Sugar-cane.—No visible growth.

Nutrient bouillon.—Slightly turbid fluid, with faint indol reaction.

Nitrate bouillon.—There is no reduction of the nitrate.

Sweet wort.—No growth.

Cane-juice.—No growth.

Milk.—Unaltered ; neutral reaction.

The Affinities of the Bacterium.

Since the organism does not form colonies on gelatine which throw out processes from the margin, it is not *Bact. gummis* to which Comes ascribes the gummosis of the vine. *Bact. apii*, said to produce a disease in celery, is larger, and grows more slowly upon potato, but otherwise there are points of similarity in the appearance of the colonies on gelatine, which, however, are white as against the yellowish colour of *Bact. vascularum*. Busse's bacteria produce gas in glucose media, and otherwise differ. *Bact. tracheiphilus*, a bacterium which causes the wilting of some of the *Cucurbitaceæ* by plugging the vessels, differs in colour (white), and by forming very ropy cultures in fluid media, especially when old. Arthur and Golden have described *Bact.*

betæ, which possibly causes gummosis of the sugar beet, with so little detail, that it cannot be compared. The same remark applies to *Bact. mori*, said by Boyer and Lambert to produce a disease (gummosis ?) of the mulberry. Of the other bacteria not associated with diseases of plants, there appear to be none described which have the general characters of this bacterium, and although Cobb's description is meagre, there is no doubt that he intended the name of *Bac. vascularum* for this organism, which he found constantly associated with the gum of affected plants.

DESCRIPTION OF PLATES.

Plate iv.

Fig. 1. Margin of an impression taken from the gum exuded from the cut vascular bundle, stained with fuchsin, and partly decolorised with alcohol (× 1000).

Fig. 2. Film from growth on cane-gelatine, stained with violet (× 1000).

Fig. 3. Film of growth on nutrient agar, stained by the night-blue method for flagella (× 1000).

Fig. 4. Colony on glucose-gelatine (× 80).

Fig. 5. Characteristic "tear-drop" growth on cane gelatine (× ½).

Plate v.

Fig. 1. Section of sugar-cane affected with gummosis, showing bacteria in large vessel, stained with dilute carbol-fuchsin (× 500).

Fig. 2. Another section showing bacteria at the margin of the contracted slime in large vessel (× 750).